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Proteasome inhibitors as anti-cancer agents.

Murray RZ, Norbury C.

Imperial Cancer Research Fund Molecular Oncology Laboratory, University of Oxford Institute of Molecular Medicine, John Radcliffe Hospital, UK.

The ubiquitin (Ub)-proteasome pathway is the major nonlysosomal pathway of proteolysis in human cells and accounts for the degradation of most short-lived, misfolded or damaged proteins. This pathway is important in the regulation of a number of key biological regulatory mechanisms. Proteins are usually targeted for proteasome-mediated degradation by polyubiquitinylation, the covalent addition of multiple units of the 76 amino acid protein Ub, which are ligated to 1-amino groups of lysine residues in the substrate. Polyubiquitinylated proteins are degraded by the 26S proteasome, a large, ATP-dependent multicatalytic protease complex, which also regenerates monomeric Ub. The targets of this pathway include key regulators of cell proliferation and cell death. An alternative form of the proteasome, termed the immunoproteasome, also has important functions in the generation of peptides for presentation by MHC class I molecules. In recent years there has been a great deal of interest in the possibility that proteasome inhibitors, through elevation of the levels of proteasome targets, might prove useful as a novel class of anti-cancer drugs. Here we review the progress made to date in this area and highlight the potential advantages and weaknesses of this approach.

Publication Types:
- Review
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PMID: 11001381 [PubMed - indexed for MEDLINE]

1: Invest New Drugs 2000 May;18(2):109-21 Related Articles, Links

Proteasome inhibition: a new strategy in cancer treatment.

Adams J, Palombella VJ, Elliott PJ.

ProScript, Inc., Cambridge, MA 02139, USA.

The ubiquitin proteasome pathway is a highly conserved intracellular pathway for the degradation of proteins. Many of the short-lived regulatory proteins which govern cell division, growth, activation, signaling and transcription are substrates that are temporally degraded by the proteasome. In recent years, new and selective inhibitors of the proteasome have been employed in cell culture systems to examine the anti-tumor potential of these agents. This review covers the chemistry of selected proteasome inhibitors, possible mechanisms of action in cell culture and the in vivo examination of proteasome inhibitors in murine and human xenograft tumor models in mice. One inhibitor, PS-341, has recently entered Phase I clinical trials in cancer patients with advanced disease to further test the potential of this approach.

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LaVallie ER, McCoy JM.

Department of Molecular Biology and Gene Expression, Genetics Institute, Cambridge, Massachusetts 02140, USA.

In recent years, Escherichia coli gene fusion expression systems have circumvented many of the problems inherent in the use of this bacterium for the production of recombinant proteins. These systems also provide a powerful means for identifying peptides or proteins with desired binding specificities. Gene fusion technology continues to expand with the introduction of new fusion partners, purification and detection tags, cleavage reagents and ways to display peptides on the surface of bacteria.

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Native quaternary structure of bovine alpha-crystallin.

Vanhoudt J, Abgar S, Aerts T, Clauwaert J.

Biophysics Research Group, Department of Biochemistry, University of Antwerp, B-2610 Antwerp, Belgium.

Alpha-crystallin is the most important soluble protein in the eye lens. It is responsible for creating a high refractive index and is known to be a small heat-shock protein. We have used static and dynamic light scattering to study its quaternary structure as a function of isolation conditions, temperature, time, and concentration. We have used tryptophan fluorescence to study the temperature dependence of the tertiary structure and its reversibility. Gel filtration, analytical ultracentrifugation, polyacrylamide gel electrophoretic analysis, and absorption measurements were used to study the chaperone-like activity of alpha-crystallin in the presence of destabilized lysozyme. We have demonstrated that the molecular mass of the in vivo alpha-crystallin oligomer is about 700 kDa (alpha(native)) while the 550 kDa molecule (alpha(37 degrees C),diluted), which is often found in vitro, is a product of prolonged storage at 37 degrees C of low concentrated alpha-crystallin solutions. We have proven that the molecular mass of the alpha-crystallin oligomer is concentration dependent at 37 degrees C. We have found strong indications that, during chaperoning, the alpha-crystallin oligomer undergoes a drastic rearrangement of its peptides during the process of complex formation with destabilized lysozyme. We propose the hypothesis that all these processes are governed by the phenomenon of subunit exchange, which is well-known to be strongly temperature-dependent.

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ACS PUBLICATIONS

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Ubiquitin fusion augments the yield of cloned gene products in Escherichia coli.

Butt TR, Jonnalagadda S, Monia BP, Sternberg EJ, Marsh JA, Stadel JM, Ecker DJ, Crooke ST.

Smith Kline & French Laboratories, Department of Molecular Pharmacology, King of Prussia, PA 19406.

Despite the availability of efficient transcription and translation signals, some heterologous gene products are not adequately expressed when introduced into prokaryotes and eukaryotes. An expression system has been established in Escherichia coli to increase the yield of cloned gene products, where the C terminus of ubiquitin was fused to the N terminus of unstable or poorly expressed proteins. Fusion of ubiquitin to yeast metallothionein or to the alpha subunit of the adenylate cyclase-stimulatory GTP-binding protein increased the yield from undetectable to 20% of the total cellular protein. A ubiquitin-N alpha-protein hydrolase has been partially purified from rabbit reticulocytes; this enzyme faithfully cleaves the junction peptide bound between the C-terminal Gly-76 of ubiquitin and the fusion protein. The increased yield of cloned gene products is very likely due to increased stability and/or more efficient translation of the fusion proteins. Possible mechanisms for the augmentation of ubiquitin fusion-protein expression in prokaryotes and eukaryotes are discussed.

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Gene fusion expression systems in Escherichia coli.

LaVallie ER, McCoy JM.

Department of Molecular Biology and Gene Expression, Genetics Institute, Cambridge, Massachusetts 02140, USA.

In recent years, Escherichia coli gene fusion expression systems have circumvented many of the problems inherent in the use of this bacterium for the production of recombinant proteins. These systems also provide a powerful means for identifying peptides or proteins with desired binding specificities. Gene fusion technology continues to expand with the introduction of new fusion partners, purification and detection tags, cleavage reagents and ways to display peptides on the surface of bacteria.

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New fusion protein systems designed to give soluble expression in Escherichia coli.

Davis GD, Elisee C, Newham DM, Harrison RG.

School of Chemical Engineering and Materials Science, University of Oklahoma, 100 East Boyd Street, Norman, Oklahoma 73019-1004, USA.

Three native E. coli proteins-NusA, GrpE, and bacterioferritin (BFR)-were studied in fusion proteins expressed in E. coli for their ability to confer solubility on a target insoluble protein at the C-terminus of the fusion protein. These three proteins were chosen based on their favorable cytoplasmic solubility characteristics as predicted by a statistical solubility model for recombinant proteins in E. coli. Modeling predicted the probability of soluble fusion protein expression for the target insoluble protein human interleukin-3 (hIL-3) in the following order: NusA (most soluble), GrpE, BFR, and thioredoxin (least soluble). Expression experiments at 37 degrees C showed that the NusA/hIL-3 fusion protein was expressed almost completely in the soluble fraction, while GrpE/hIL-3 and BFR/hIL-3 exhibited partial solubility at 37 degrees C. Thioredoxin/hIL-3 was expressed almost completely in the insoluble fraction. Fusion proteins consisting of NusA and either bovine growth hormone or human interferon-gamma were also expressed in E. coli at 37 degrees C and again showed that the fusion protein was almost completely soluble. Starting with the NusA/hIL-3 fusion protein with an N-terminal histidine tag, purified hIL-3 with full biological activity was obtained using immobilized metal affinity chromatography, factor Xa protease cleavage, and anion exchange chromatography. Copyright 1999 John Wiley & Sons, Inc.

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J Biol Chem. 1997 Jan 24;272(4):2578-82.
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Purification, structure and in vitro molecular-chaperone activity of Artemia p26, a small heat-shock/alpha-crystallin protein.

Liang P, Amons R, Macrae TH, Clegg JS.

Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada.

Encysted brine-shrimp gastrulae bring their metabolism to a reversible standstill during diapause and quiescence, demonstrating a remarkable resistance to unfavourable environmental conditions. For example, mortality of Artemia embryos under normal temperature and hydration is very low, even after two years of anoxia, and embryos commonly experience complete desiccation as part of their developmental program. Previous evidence from our laboratories indicated that p26, an abundant low-molecular-mass cyst-specific protein capable of translocation into the nucleus, may have a protective function in Artemia cysts. p26 was purified to apparent homogeneity and a continuous sequence of 141 of its amino acids was determined by peptide sequencing, revealing that it is a member of the small-heat-shock/alpha-crystallin family of proteins. As determined by molecular-sieve chromatography and sucrose-density-gradient centrifugation, native p26 is a multimer of about 27 monomers with a molecular mass of approximately 700 kDa. Inactivation of citrate synthase was less when the enzyme was heated in the presence rather than the absence of p26. Additionally, the renaturation of heat-inactivated citrate synthase was promoted by p26. These results indicated that p26 possesses molecular-chaperone activity, a property of other small heat-shock/alpha-crystallin proteins. Our findings demonstrate that p26 has the potential to protect the the macromolecular components of Artemia embryos, either as they encyst or upon exposure to environmental extremes. Protection may depend upon the ability of p26 to function as a molecular chaperone.

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The synthesis of a small heat shock/alpha-crystallin protein in Artemia and its relationship to stress tolerance during development.

Liang P, MacRae TH.

Department of Biology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada.

Fertilized oocytes of the brine shrimp Artemia franciscana undergo either ovoviviparous or oviparous development, yielding free-swimming larvae (nauplii) or encysted gastrulae (cysts), respectively. Encystment is followed by diapause, wherein metabolism is greatly reduced; the resulting cysts are very resistant to extreme stress, including desiccation and long-term anoxia. The synthesis of p26, a small heat shock/alpha-crystallin protein produced only in oviparously developing Artemia, is shown in this paper to be transcriptionally regulated. A p26 mRNA of about 0.7 kb was detected on Northern blots in the second day after oocyte fertilization. It peaked as embryos encysted and declined rapidly when activated cysts resumed development. The appearance of p26 protein, as indicated by immunoprobing of Western blots, followed mRNA by 1 day; it also increased as encystment occurred but remained constant during postgastrula development of cysts. However, p26 underwent a marked reduction during emergence of nauplii and could not be detected in cell-free extracts of second-instar larvae. p26 entered nuclei of encysting embryos soon after synthesis and was localized therein as late as instar II, when it was restricted to a small set of salt gland nuclei. First-instar larvae derived from cysts were more thermotolerant than larvae that had developed ovoviviparously, but synthesis of p26 was not induced by heat under the experimental conditions employed. Additionally, transformed bacteria synthesizing p26 were more thermotolerant than bacteria that lacked the protein. The results support the proposal that p26, a developmentally regulated protein synthesized during embryo encystment, has chaperone activity in vivo and protects the proteins of encysted Artemia from stress-induced denaturation. Copyright 1999 Academic Press.

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Alpha-crystallin as a molecular chaperone.

Derham BK, Harding JJ.

Nuffield Laboratory of Ophthalmology, University of Oxford, UK.

The role of alpha-crystallin as a molecular chaperone may explain how the lens stays transparent for so long. Alpha-crystallin prevents the aggregation of other lens crystallins and proteins that have become unfolded by "trapping" the protein in a high molecular weight complex. It also protects enzyme activities. The substrate protein may interact while in a molten globule state. Alpha-crystallin predominantly binds to proteins very early in the denaturation pathways. The amphiphilic nature of alpha-crystallin, a polar C-terminal-region and a hydrophobic N-terminal-region are all essential for chaperone function. The flexible C-terminal extension maintains solubility and can bind to opposing charged residues of unfolding proteins. Hydrophobic regions in the N-terminal region then hold the unfolded protein. Specific areas important for chaperone binding and function have been identified throughout the N-terminal-region, connecting peptide and C-terminal extension. After a substantial amount of chemical data and models, cryo-EM images of alpha-crystallin have confirmed a variable 3D surface with a hollow interior. Alpha-crystallin taken from the lens nucleus shows an age-dependent decrease in chaperone function. High molecular weight aggregates and alpha-crystallin found within the nucleus from clear and cataract lenses have reduced chaperone function. Post-translational modifications, known to occur during ageing, such as glycation, carbamylation, oxidation, phosphorylation and truncation cause a decrease in chaperone function. Alpha-crystallin is expressed outside the lens. AlphaB-crystallin can be induced by heat shock in many tissues where it is translocated from cytoplasm to nucleus. Increased expression of alphaB-crystallin has been seen in many pathological states. Conformational disorders, including cataract may have a common aetiology and potentially a common therapy.

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Overexpression of a cytosolic chaperone to improve solubility and secretion of a recombinant IgG protein in insect cells.

Ailor E, Betenbaugh MJ.

Department of Chemical Engineering, The Johns Hopkins University, Baltimore, Maryland 21218, USA.

The secretion of heterologous IgG proteins in the baculovirus-insect cell expression system is accompanied by substantial insoluble immunoglobulin in the infected cells. The accumulation of these insoluble forms suggests a limitation in the processing and secretory pathway of the infected cells. As a result, cytosolic hsp70 chaperones, which are known to associate and prevent aggregation of polypeptides in vitro, have been coexpressed in the infected cells. The hsp70 protein coprecipitated with the immunoglobulin to indicate the formation of a specific hsp70-immunoglobulin complex in vivo. Immunoblot and pulse chase studies indicated that coexpression of hsp70 increased intracellular immunoglobulin solubility. Metabolic labeling experiments revealed that hsp70 increased secreted immunoglobulin levels after several days infection as compared to infection with control baculoviruses. Pulse chase studies indicated that hsp70 increases the solubility of immunoglobulin precursors that are then processed and assembled into the complete antibody oligomer. A comparison of the action of cytosolic hsp70 chaperone to the endoplasmic reticulum chaperone BiP suggests sequential action in which hsp70 increases the solubility of preprocessed immunoglobulin, while BiP enhances the solubility of processed immunoglobulin chains. Copyright 1998 John Wiley & Sons, Inc.

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Alpha-crystallin as a molecular chaperone.

Derham BK, Harding JJ.

Nuffield Laboratory of Ophthalmology, University of Oxford, UK.

The role of alpha-crystallin as a molecular chaperone may explain how the lens stays transparent for so long. Alpha-crystallin prevents the aggregation of other lens crystallins and proteins that have become unfolded by "trapping" the protein in a high molecular weight complex. It also protects enzyme activities. The substrate protein may interact while in a molten globule state. Alpha-crystallin predominantly binds to proteins very early in the denaturation pathways. The amphiphilic nature of alpha-crystallin, a polar C-terminal-region and a hydrophobic N-terminal-region are all essential for chaperone function. The flexible C-terminal extension maintains solubility and can bind to opposing charged residues of unfolding proteins. Hydrophobic regions in the N-terminal region then hold the unfolded protein. Specific areas important for chaperone binding and function have been identified throughout the N-terminal-region, connecting peptide and C-terminal extension. After a substantial amount of chemical data and models, cryo-EM images of alpha-crystallin have confirmed a variable 3D surface with a hollow interior. Alpha-crystallin taken from the lens nucleus shows an age-dependent decrease in chaperone function. High molecular weight aggregates and alpha-crystallin found within the nucleus from clear and cataract lenses have reduced chaperone function. Post-translational modifications, known to occur during ageing, such as glycation, carbamylation, oxidation, phosphorylation and truncation cause a decrease in chaperone function. Alpha-crystallin is expressed outside the lens. AlphaB-crystallin can be induced by heat shock in many tissues where it is translocated from cytoplasm to nucleus. Increased expression of alphaB-crystallin has been seen in many pathological states. Conformational disorders, including cataract may have a common aetiology and potentially a common therapy.

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